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Applicants:	Andrew VAILLANT et al.
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For:	ANTIVIRAL OLIGONUCLEOTIDES
Art Unit:	1648
Examiner:	Sharon L. Hurt
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DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Jean-Marc Juteau, do hereby declare and state as follows:

1. I received the degrees of Bachelor (B.Sc.) of Biology from Montreal University in 1985, Master (M.Sc.) of Microbiology and Immunology from Montreal University in 1988, and Doctor of Philosophy (Ph.D.) of Microbiology and Immunology from Laval University in 1991.
2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
3. I am a founder since 1999 of REPLICor Inc. and Senior Vice President since 2002.
4. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.

5. I am an inventor in the present application; I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 10/661,403 entitled "ANTIVIRAL OLIGONUCLEOTIDES", including the claims.
6. I have also read and understood the latest Official Action from the PTO dated February 22, 2006. In this Office Action, claims 28 and 29 were rejected for lack of enablement under 35 U.S.C. §112, first paragraph.
7. The following experiments had been performed in Jan-Feb 2006 (simian immunodeficiency virus), Sept 2004 (Friend leukemia virus), April 2005 & July 2006 (influenza virus), Nov. 2004 (respiratory syncytial virus), April 2005 (herpes virus-2), Dec. 2005 (cytomegalovirus), Nov. 2004 (Ebola virus) and Sept. 2004 (vaccinia virus) under the supervision of Andrew Vaillant (inventor on this invention) and myself, to obtained results with eight (8) animal models showing the *in vivo* antiviral activity of sequence independent oligonucleotides of the present invention. These *in vivo* models of viral infection are recognized models that can be used for the demonstration of a drug treatment activity. These *in vivo* treatment models cover different viruses, different viral families, RNA and DNA viruses, different pathogenic aspects, different routes of administration, different drug dosages, different formulations and different ONs.

The following experiments were conducted to evaluate the therapeutic antiviral activity of sequence independent oligonucleotides *in vivo*.

a) **Simian immunodeficiency virus**

The infection of a rhesus macaque with the simian immunodeficiency virus (SIV) is considered to be the animal model to most closely approximate the infection of humans with HIV. SIV, HIV-1 and HIV-2 are retroviruses showing extensive homology in their genomes. The rhesus macaque SIV model closely mimics the progression of human HIV infection.

To establish the suitability of an oligonucleotide (ON) as a therapy for HIV infection in humans, we tested its ability to reduce serum viral titers in a macaque chronically infected with SIV. A phosphorothioated 40randomer (REP 2006) was administered by continuous intravenous administration through a cannula inserted in the jugular vein. The sodium salt of REP 2006 was dissolved in normal saline (0.9% NaCl) at the following concentrations: 0.112mg/ml, 0.225mg/ml, 0.450mg/ml, 0.675mg/ml, 0.90mg/ml and 1.13mg/ml. After dissolution, the REP 2006/normal saline solution was heated to 65 deg C for 15 min and then allowed to cool to room temperature. The solutions were then filter sterilized through a 0.22uM cellulose acetate filter and transferred to sterile 50ml falcon tubes. Formulations were stored at -80 deg C until required. Prior to administration, solutions were thawed to room temperature and then heated to 37 deg C for 5 minutes. 50cc of prewarmed REP 2006 solution was then loaded into the syringe of a programmable infusion pump. Infusion rates were controlled according to the following schedule: 10cc/h for 2h → 0.5cc/h for 10h → 10cc/h for 2h →

0.5cc/h for 10h. Serum SIV titers were monitored each week using bDNA SIV assay.

As shown in Table 1, a macaque showed a continuous drop in viral titer during the 11 week treatment, demonstrating the effectiveness of a sequence independent ON in lowering the SIV titers when administered by daily continuous infusion.

Table 1
Reduction in serum titer in a REP 2006 treated, SIV-infected macaque

WEEK	REP 2006 DOSE (total mg/kg/day)	SIV serum titer (copies / ml)
1	0	1479300 (pre-treatment titer)
2	1	2784300
3	1	1245900
4	2	1070400
5	2	826304
6	4	648507
7	4	581487
8	6	477427
9	8	639965
10	8	575179
11	10	548428
12	10	427217

This data demonstrates the anti-retroviral activity *in vivo* of the sequence independent oligonucleotides of the present invention.

b) **Friend Leukemia Virus**

The Friend Leukemia Virus (FLV) is an immunosuppressive retrovirus such as HIV. Although the FLV model is not as close to HIV as is the SIV model, it is a well established model for studying genetic resistance to retroviruses infection.

Mice received FLV innoculum on day 0 by IV injection. Mice received 10mg/kg of a phosphorothioated 40randomer (REP 2006) by a once daily 100ul bolus subcutaneous injection on days -2, -1, 0, 1, 2, 3, 4, 5. REP 2006 was prepared by dissolving the GMP sodium salt of REP 2006 in 5% dextrose. This solution was heated to 65 deg C for 15 minutes, then cooled to room temperature. The DNA content of the REP 2006 solution was adjusted to be 0.15 mM (using A260) by additional dilution with the appropriate amount of 5% dextrose. The final solution was filter sterilized through a 0.22 um cellulose acetate filter and stored at -20 deg C until use. Aliquots were warmed to 37 deg C prior to administration. Spleens were harvested on day 6 and infection was monitored by fluorescence assisted cell sorting (FACS) to detect the percentage of infected splenocytes using a monoclonal antibody against CD34.

As shown in Table 2, treated mice showed a 2.5 fold reduction of FLV infected splenocytes compared to untreated animals.

Table 2
Summary of statistical data (percentage of infected splenocytes)

Parameter	Placebo (5% dextrose)	10mg/kg/day REP 2006
Mean	29.3	11.27
N	4	4
Std. dev.	6.99	6.22
Maximum	22.8	6.3
Minimum	38.7	19.3
Lower 95% CI	18.17	1.37
Upper 95% CI	40.43	21.179

This data demonstrates the anti-retroviral activity *in vivo* of the sequence independent oligonucleotides of the present invention.

c) **Influenza virus**

The mouse influenza infection model has been recognized to be predictive of clinical efficacy of antiviral drugs in several instances. The model shows mainly infection in the lung.

A phosphorothioated 40randomer ON (REP 2006) or a phosphorothioated 40mer polyC (REP 2031) were administered daily to mice either by the aerosol (inhalation) or parenteral (subcutaneous or intraperitoneal injection) either before or after infection and for 2 days after infection of animals with human influenza strains (mouse adapted). REP 2006 was prepared as a 100 or 10mg/ml solution dissolution of the sodium salt of REP 2006 into water. The solution was heated to 65 deg C for 15 minutes, then allowed to cool to room temperature. Cooled solutions were then filter sterilized using a 0.22um cellulose acetate filter. Solutions were stored at -20deg C until use. Samples were thawed to room temperature and then heated to 37 deg C for 5 minutes before administration. REP 2006 solution was added to the reservoir of a Aerotech II nebulizer when was then used to generate REP 2006 aerosol using air delivered to the nebulizer at a flow rate of 10 L/min @ 15 PSI. REP 2006 aerosol was directed using standard medical aerosol tubing into the cage where the mice were situated. Aerosol exposure was allowed to continue for 30 min which used approximately 10 ml of REP 2006 solution. Antiviral activity was assessed by the determination of viral titers in the lungs.

Table 3

Effect of aerosol and parenteral administration of ONs in influenza infected mice.

a)

REP 2006 dose and route of administration	Influenza A lung titer (log_{10}/g lung)
0 (placebo control)	5.8
2X10mg/ml/day (10 ml aerosol) before infection	3.3
2X100mg/ml/day (10ml aerosol) 24h after infection	1.1
20mg/kg (intraperitoneal) 24h after infection	3.2
20mg/kg (subcutaneous) 24h after infection	3.9

b)

Dose, time of administration and ON	Influenza A lung titer (log_{10}/g lung) 4 days after infection
0 (placebo control)	7.04
10 min of 10mg/ml aerosol, 1 day before infection, REP 2006	6.56
10 min of 10mg/ml aerosol, 1day before infection, REP 2031	6.61

This data shows that ONs of this invention are an effective *in vivo* treatment in influenza infections. The administration of ONs was well tolerated in all dosing regimens listed above.

d) **Respiratory syncytial virus**

To date, the only animal model which has been predictive of antiviral activity against respiratory syncytial virus (RSV) infections in humans has been RSV infection in the cotton rat. While the progression of infection in this model is slower than in mice (or in humans), it has been demonstrated that the viral receptor utilization by RSV in the cotton rat is highly similar to that in humans.

A phosphorothioated 40randomer (REP 2006) was administered daily to cotton rats by aerosol inhalation (using the exact formulation procedures described for aerosol administration of REP 2006 to mice in the example for influenza A above) starting 1 day prior to infection with a human stain of RSV (rat adapted) and continuing for 2 days after infection. Antiviral activity was assessed by the determination of viral titers in the lungs.

Table 4
Effect of aerosol administration of ONs in RSV infected cotton rats.

REP 2006 dose and route of administration	RSV lung titer (log_{10}/g lung) 4 days after infection
0 (placebo control)	3.9
2X100mg/ml/day (10ml aerosol) 24h after infection	2.8

These results show that ONs of this invention are an effective *in vivo* treatment against RSV infection. The administration of ONs was well tolerated in this model.

e) **Herpes virus-2**

The vaginal human herpes simplex-2 (HSV-2) transmission model in mouse has been demonstrated to be useful in predicting clinical efficacy of topical microbicides against the transmission of HSV-2 (genital herpes).

The antiviral activity of ONs was tested in a topical (vaginal) model of transmission of HSV-2. A phosphorothioated 40randomer (REP 2006) or a phosphorothioated 40mer polyC (REP 2031) were applied topically as a single

application in the vagina before vaginal infection with the virus. The sodium salts of REP 2006 or REP 2031 were dissolved in normal saline (0.9% NaCl) to 100mg/ml, heated to 65 deg C for 15 min then cooled to room temperature. Cooled solutions were then filter sterilized with a 0.22um cellulose acetate filter. Solutions were stored frozen at -20 deg C until use. Before application, samples were warmed to 37 deg C for 5 min. Antiviral activity was monitored by viral titration in the vagina.

Table 5
ONs are effective topical agents against HSV-2 transmission

PS-ON dose	% of animals protected from transmission
0 (placebo control)	0/12
100mg/ml REP 2006	8/12
100mg/ml REP 2031	12/12

This data shows that ONs of this invention are effective antiviral agents in treating *in vivo* CMV infection. In this *in vivo* model, administration of the compound was well tolerated.

f) **Cytomegalovirus**

The mouse model of cytomegalovirus (CMV) infection is a well established model for the study of systemic and organ, such as liver and spleen, infection. It has been used for testing activity of several treatments.

The activity of ONs was tested in an animal model of human CMV. A phosphorothioated 40 randomer (REP 2006) or phosphorothioated 40mer polyC

(REP 2031) or a phosphorothioated-2'O-methyl 40randomer (REP 2107) were administered parenterally (as a daily 1ml intraperitoneal injection) for 2 days prior to infection, during infection and for 3 days after infection. The sodium salts of REP 2006, REP 2031 or REP 2107 were dissolved in normal saline (0.9% NaCl) to a concentration of 0.5mg/ml. Solutions were then heated to 65 deg C for 15 minutes, cooled to room temperature and filter sterilized using a 0.22um cellulose acetate filter. Sterile solutions were stored frozen at -20 deg C and thawed and warmed to 37 deg C prior to administration Antiviral activity was monitored by viral titration in the liver.

Table 6
ONs are effective agents against systemic CMV infection

ON dose	Liver titer (log10/ml tissue)
0 (placebo control)	2.9
20mg/kg/day REP 2006	1.1
20mg/kg/day REP 2031	0.7
20mg/kg/day REP 2107	0.9

These results show that ONs of this invention are effective antiviral agents in treating *in vivo* CMV. In this *in vivo* model, administration of the compound was well tolerated.

g) **Ebola virus**

The mouse model of Ebola virus infection is a lethal model used to study the infectivity of the virus and has been used to assay treatments such as monoclonal antibodies.

A phosphorothioated 40randomer (REP 2006) activity against Ebola Zaire was tested by daily intraperinoneal injection for 12 days in mice which had received a lethal injection of human Ebola Zaire virus. REP 2006 was prepared by dissolving the GMP sodium salt of REP 2006 in normal saline (0.9% NaCl). This solution was heated to 65 deg C for 15 minutes, and then cooled to room temperature. The DNA content of the REP 2006 solution was adjusted to be 0.15 mM (using A260) by additional dilution with the appropriate amount of normal saline. The final solution was filter sterilized through a 0.22 um cellulose acetate filter and stored at -20 deg C until use. Aliquots were warmed to 37 deg C prior to administration.Antiviral activity was assessed by the survival of the animals 2 weeks after all placebo animals had died (usually 10-12 days after infection).

Table 7
ONs are effective agents against Ebola Zaire infection in mice

REP 2006 dose	Survival
0 (placebo control)	0/8
20mg/kg/day (12days)	8/8

These results show that ONs of this invention are effective antiviral treatment against Ebola infection *in vivo*. In this model, the treatment was well tolerated.

h) **Vaccinia virus**

The vaccinia virus is the human vaccine for smallpox and can be used in a mouse model as a surrogate for smallpox virus. Due to the extremely lethal nature of smallpox and its lack of an appropriate animal reservoir, direct testing of

compounds against smallpox infections in chimpanzees cannot be performed. The model has been used to test therapeutic activity of antiviral compounds.

A phosphorothioated 40randomer (REP 2006) was given by 13 daily subcutaneous injections to mice which had received a lethal vaccinia infection. REP 2006 was prepared by dissolving the GMP sodium salt of REP 2006 in 5% dextrose. This solution was heated to 65 deg C for 15 minutes, then cooled to room temperature. The DNA content of the REP 2006 solution was adjusted to be 0.15 mM (using A260) by additional dilution with the appropriate amount of 5% dextrose. The final solution was filter sterilized through a 0.22 um cellulose acetate filter and stored at -20 deg C until use. Aliquots were warmed to 37 deg C prior to administration. Antiviral activity was monitored by the survival of animals 2 weeks after the normal lethal period (10 days).

Table 8
REP 2006 has antiviral activity against Vaccinia infection *in vivo*.

REP 2006 dose	Survival
0 (placebo control)	0/10
12mg/kg/day (12days)	2/10

This data shows that ONs of this invention have antiviral therapeutic activity against vaccinia and other poxviruses infection *in vivo*. In this model, administration was well tolerated.

8. The results presented above and produced according to the teaching of the present invention clearly proves that that the present invention has clinical relevance and

in addition, that the *in vitro* results disclosed in the present application do not diverge from *in vivo* responses. The antiviral activity of the sequence independent oligonucleotides of the present invention is demonstrated in eight (8) different *in vivo* models of viral infections.

9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed  Dated: July 26, 2006

Jean-Marc Juteau

Curriculum vitae

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Age: 42

Status: Married, three kids

Language spoken and written: French and English

EXPERIENCE

01-2002 - today

Senior Vice-President and Founder, REPLICor Inc., Laval.
Biopharmaceutical company developing antiviral and anticancer drugs.

Responsibilities:

- Science development.
  Day to day contact with CSO, scientific input.
- In charge of intellectual property portfolio.
  Patent writing, strategy, management.

02-1999 – 01-2002

CEO and founder, REPLICor Inc., Laval.

Responsibilities:

- Science development
- In charge of financing
  Instrumental in raising $2.5M in equity and loan
- In charge of licensing and contract agreement
  Negotiation of licenses and contracts with universities

02-1996 to 02-1999

Officer, Office of Technology Transfer, McGill University, Montreal.

Responsibilities:

- Agreement management and negotiation
  License, research, option, confidentiality, material distribution.
- Spin-off company projects
  Set-up of spin-off company, contact with investors, business plan.

03-94 to 02-96

Product Manager, Iso Tech Design, Laval
Company developing and marketing micro-environments for pharma applications.

Responsibilities:

- Microbiology quality control..

- Distributor formation
  Contacts: Baxter Health Care, VWR, Khulman Tech., E.S.I. FluFrance, Liberty Clean Rooms, Millipore.

91 à 10-93
Director and Co-founder, DIAGNOGENE inc., R&D in biotechnology, Ste-Foy
Responsibilities: Financial and research administration, representation.

RESEARCH TRAINING

09-92 à 11-93
Post-doctoral scientist, **INRS-santé**, Pointe-Claire
Project: In-vitro mutagenesis of 4-chlorobenzoate dehalogenase in *Pseudomonas sp.* CBS3.

08-91 à 09-92
Post-doctoral scientist, **Institut de Recherches Cliniques de Montréal**
Project: Cloning et characterization of a cardiac specific transcription factor.

11-90
Training in molecular modeling, Department of Molecular and Cell Biology, **University of Connecticut.**

05-88 to 06-88
Workshop on DNA technologies: Sequence and in-vitro mutagenesis, **University of North-Carolina**, Chapel Hill, NC.

EDUCATION

87-91
Doctorate (Ph.D)., Microbiology and Immunology, **Laval University.**
Molecular biology, epidemiology and structure-function analysis of the ROB-1 ß-lactamase.

85-87
Master (M.Sc)., Microbiology and Immunology, **Montreal University and Hôtel-Dieu Hospital.**
Granulocytar function in recurrent vaginitis.

82-85
Bachelor (B.Sc.)., Biology, **Montreal University.**

BOARD MEMBERSHIP

2005- today
Member of the Montreal Life Science Committee.

2004- today
President of the Alumni Association of Montreal Clinical Research Institute.

SCHOLARSHIP, AWARD and PRIZES

Industrial Design Prize 1995 from the Design Institute (received in team for a micro-environment)
Institut National de la Recherche Scientifique (INRS) Fellowship, 1992-93.
Medical Research Council (MRC)Fellowship, 1992.
Fonds de la Recherche en Santé du Québec (FRSQ) Studentship, 1989-90-91.
Fonds pour la Formation des Chercheurs et l'Aide à la Recherche (FCAR) Studentship, 1988-89.
Canlab Prize from l'Association des Microbiologistes du Québec, 1989.

AUTHORSHIP

Patent filings:	20
Scientific articles:	10
Posters and oral presentations:	30

Vaillant A, Juteau JM, Lu H, Liu S, Lackman-Smith C, Ptak R, Jiang S. Phosphorothioate oligonucleotides inhibit human immunodeficiency virus type 1 fusion by blocking gp41 core formation. Antimicrob Agents Chemother. 2006 Apr;50(4):1393-401.

Kocisko DA, Vaillant A, Lee KS, Arnold KM, Bertholet N, Race RE, Olsen EA, Juteau JM, Caughey B. Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. Antimicrob Agents Chemother. 2006 Mar;50(3):1034-44.

Moaddel R, Price GB, Juteau JM, Leffak M, Wainer IW. The synthesis and initial characterization of an immobilized DNA unwinding element binding (DUE-B) protein chromatographic stationary phase. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 Jun 25;820(2):197-203.

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Ahmad D, Fraser J, Sylvestre M, Larose A, Khan A, Bergeron J, Juteau JM, Sondossi M. Sequence of the bphD gene encoding 2-hydroxy-6-oxo-(phenyl/chlorophenyl)hexa-2,4-dienoic acid (HOP/cPDA) hydrolase involved in the biphenyl/polychlorinated biphenyl degradation pathway in Comamonas testosteroni: evidence suggesting involvement of Ser112 in catalytic activity.Gene. 1995 Apr 14;156(1):69-74.

Juteau JM, Billings E, Knox JR, Levesque RC. Site-saturation mutagenesis and three-dimensional modelling of ROB-1 define a substrate binding role of Ser130 in class A beta-lactamases. Protein Eng. 1992 Oct;5(7):693-701.

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Juteau JM, Cote S, Levesque RC. Systematic site-saturation mutagenesis of ROB-1 beta-lactamase: efficiency of T4 polymerase and oligonucleotide synthesis. Biotechniques. 1991 Oct;11(4):460-2.

Juteau JM, Sirois M, Medeiros AA, Levesque RC. Molecular distribution of ROB-1 beta-lactamase in Actinobacillus pleuropneumoniae. Antimicrob Agents Chemother. 1991 Jul;35(7):1397-402.

Juteau JM, Levesque RC.Sequence analysis and evolutionary perspectives of ROB-1 beta-lactamase. Antimicrob Agents Chemother. 1990 Jul;34(7):1354-9.